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Review 综 述

基于益生菌口服药物递送系统的研究进展

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摘　要：随着当今人们对健康的不断重视，益生菌因其安全、有益于肠道健康的特点逐渐进入人们的视野，并成为食品、医药等领域的热点。然而在口服给药方面，选择使用一种安全、方便、稳定的载体是一个难题。以益生菌为载体的口服给药系统具有优异的安全性和稳定性，同时又可以保护被递送药物通过复杂的体内环境而不受破坏。本文综述了5种常见的基于益生菌的口服药物递送方式：芽孢表面展示、酵母微胶囊、重组益生菌表达、细菌样颗粒和细菌影，并详细介绍了它们各自的结构和优缺点，为口服递送载体的开发奠定了坚实的理论基础。

关键词：口服给药；益生菌；芽孢；递送载体

Research progress in oral delivery systems with probiotics as carriers

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Abstract: With the increasing emphasis on health, probiotics have garnered increasing attention due to their safe and beneficial features for intestinal health and have become a hot spot in the fields of food and medicine. In terms of oral drug delivery, it is difficult to select a safe, convenient, and stable carrier. Probiotics can be used as carriers to deliver drugs through

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the complex *in vivo* environment without damage and have excellent safety and stability. This review describes the structures, advantages, and disadvantages of five common probiotic-based oral drug delivery approaches: display on spore surface, yeast microcapsules, recombinant probiotic expression, bacterium-like particles, and bacterial ghost, aiming to provide options for the application of oral delivery carriers.

Keywords: oral drug delivery; probiotics; spore; delivery carrier

2001 年，世界卫生组织(World Health Organization, WHO)首次将益生菌定义为“摄入足够的量时可以给予宿主健康益处的一类活的微生物”[1]。益生菌包括许多不同种属的微生物，例如乳酸杆菌(*Lactobacillus*)、链球菌(*Streptococcus*)、乳球菌(*Lactococcus*)、双歧杆菌(*Bifidobacterium*)、丙酸杆菌(*Propionibacterium*)、益生芽孢杆菌(*Bacillus*)和大肠杆菌(*Escherichia coli*) Nissle1917 等。此外，一些酵母，例如酿酒酵母(*Saccharomyces cerevisiae*)、卡氏酵母(*Saccharomyces carlsbergensis*)、布拉氏酵母(*Saccharomyces boulardii*)和黑曲霉(*Aspergillus niger*)、米曲霉(*Aspergillus oryzae*)等真菌也被认为是益生菌[2]。益生菌既可以是天然存在的微生物，也可以是由于某些特殊效应而导致基因改变的微生物[3]。过去十多年间，益生菌因其健康效益受到越来越多的关注。许多研究表明，益生菌具有调节人体肠道菌群、治疗癌症、缓解炎症性肠病、治疗胃肠道疾病等健康效益[4-7]。时至今日，益生菌及其制剂已经广泛应用于食品、医药、农业、渔业和日化等领域中[8-12]。

口服给药是传统给药方式之一，因其方便性和优秀的患者依从性，口服给药已经成为许多疾病治疗方式的主要给药途径[13-14]。尽管如此，许多药物存在着稳定性差、溶解度低和靶向作用弱等问题，直接口服的生物利用度较低、治疗效果较差[15]。为应对此困境，口服给药通常会使用相关载体，不仅可以提高口服药物的稳定性，增强口服药物的生物利用度和治疗效果，还可以维持药物浓度在合适的范围内，降低其副作用[16]。因此，如何开发和利用新兴的口服递送载体已经成为了一大研究热门。伴随着纳米医药技术的进步，现在的口服递送载体的种类纷繁复杂，例如脂质体、纳米颗粒、纳米复合材料、微胶囊、树状体和细胞载体等已被广泛应用于口服药物递送[17-20]。

益生菌因其可以定殖在肠道黏膜表面、不引起强烈的免疫反应，以及可以发挥一些积极的免疫调节功能的特点[21]，在用作口服递送载体的方面具有很好的潜力。本课题组 Wang 等[22]的研究证明了一种植物乳杆菌 MA2 具有很好的在肠道内定殖的能力，其可以调节肠道微生物群和阿尔茨海默病(Alzheimer’s disease, AD)代谢紊乱，能够改善 AD 大鼠的认知缺陷和焦虑样行为。然而，许多的益生菌，例如乳酸菌和益生芽孢杆菌、大肠杆菌 Nissle1917 (*EcN*)等，已经被广泛开发用作各类药物递送途径的载体[23-26]。本综述聚焦于益生菌作为口服递送载体的几种不同形式应用，如微胶囊、重组载体、芽孢表面展示、细胞样颗粒和细菌影，并介绍各装载药物模式与其特点(表 1)。

1 芽孢杆菌表面展示系统

芽孢杆菌是一类具有较大尺寸、需氧或兼性厌氧的革兰氏阳性菌，其最大的特点是能够产生无代谢活性的芽孢[27]。芽孢，又称为内生孢子，

表 1　口服递送载体的模式与特点

Table 1　Patterns and characteristics of oral delivery vehicles

Oral delivery vehicles	Drug loading mode	Characteristic
Bacillus surface display system	Recombination surface display	No need to produce antigens; different antigens can be displayed
	Non-recombinant surface display	Highly efficient; exhibits antigen in its natural form; requires cleavage to exhibit antigen
Yeast microcapsule system	Electrostatic adsorption Non-covalent force modification Passive diffusion/hydrophobicity Layer-by-layer synthesis (LBL) Post-derivatization combination	Protects drugs from destruction; accurately targets drugs; efficiently loads all types of drugs; safe and non-toxic
Recombinant probiotic expression system	Surface display	Stable binding; no need to penetrate cells; high efficiency
	Secrete	High delivery efficiency; avoids drug toxicity to cells
	Intracellular	Avoids environmental destruction of drugs; toxic to cells
Bacterium-like particles, BLPs	Surface display	Safe and stable; high drug loading; insignificant therapeutic immunization effect
Bacterial ghosts, BGs	Anchors and fills in internal spaces	Safe and stable; efficient targeting; its structure decreases drug concentration

其可以在休眠状态下长期存活，并且可以抵抗高温、缺水、缺乏营养、高酸碱度和有毒化学物质等极端恶劣条件[28-30]。

芽孢的结构在芽孢的抗性中起着关键的作用。如图 1 所示，芽孢的结构内向外看依次为核芯、内膜、皮层、外膜、芽胞内壳、芽孢外壳和芽孢外壁(但并非所有种类的芽孢都有芽孢外壁)。芽孢的结构与其抗性的具体关系在 Setlow 的研究中已经进行了详细的综述[31]。因其特殊结构与优秀的抗性，使得芽孢可以在口服后穿过胃部并在肠道中存活和发芽[32]。芽孢杆菌具有较大的疏水性比表面积，因此可以通过吸附或共价结合的方式装载大量的疏水性药物[33]。芽孢杆菌的芽孢不仅易于进行基因改造，而且不具有致病性[34-35]。基于上述的特性，使得芽孢杆菌的芽孢成为一种良好的口服药物递送平台。

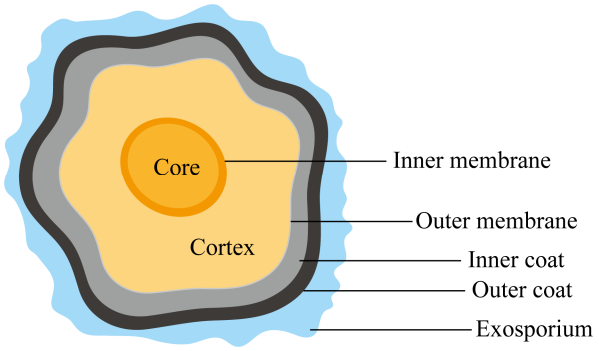

图 1　芽孢杆菌芽孢的结构示意图　不同层的尺寸未按比例绘制

Figure 1　Schematic structure of *Bacillus* spores. Dimensions of different layers not drawn to scale.

芽孢杆菌表面展示系统可以分为重组芽孢杆菌表面展示型和非重组芽孢杆菌表面展示型。重组芽孢杆菌表面展示型是通过将目标基因和编码芽孢衣壳蛋白(称为锚蛋白)的基因导入其自身的启动子中，然后宿主菌株在苛刻的培养环境中诱导芽孢形成，最后在宿主菌株芽孢表面表

达外源蛋白或多肽[36]。Yang 等[37]将衣壳蛋白 CotB) 和曲霉毒素的甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase of *H. contortus*, HcGAPDH) 的基因融合进质粒 pDG364 中，使得 HcGAPDH 在枯草芽孢杆菌芽孢表面成功表达。Chen 等[38]通过同源双交叉重组将由锚蛋白 CotB 和冠状病毒多肽 HR2 编码基因组成的融合基因通过质粒 pDG364 插入到枯草芽孢杆菌中，成功设计出一种芽孢表面展示 HR2 肽的猫冠状病毒(feline coronavirus, FCoV)疫苗。Sun 等[34]将华支睾吸虫副肌球蛋白 CsPmy 的编码序列克隆到 PEB03-CotC 质粒里，并将其转化到枯草芽孢杆菌 WB600 菌株中，成功构建出一个 CsPmy 芽孢表面展示平台 B.s-CotC-CsPmy。

非重组芽孢杆菌表面展示型是通过芽孢表面与外源蛋白的静电与疏水作用力或通过交联剂将外源蛋白吸附于芽孢表面[39]。宋庆龄[40]在芽孢表面修饰脱氧胆酸(deoxycholic acid, DA)并装载盐酸阿霉素(doxorubicin hydrochloride, DOX)和索拉菲尼(sorafenib, SOR)，构建出一种口服自主纳米药物“生成器”。Yin 等[33]将姜黄素分别与芽孢杆菌的外壳和叶酸共价连接，开发出了一种新型口服结肠靶向载体(SPORE-CUR-FA)用于治疗结肠癌。值得注意的是，灭活的芽孢也可以稳定表面吸附蛋白质，其免疫保护效果仍与未灭活芽孢相当。Song 等[41]研究发现，灭活的芽孢仍然能够与甲型流感(H5N1)病毒粒子结合，并且可以有效增强口服后产生的免疫反应。表 2 罗列出了更多重组和非重组的芽孢杆菌表面展示系统的应用。

芽孢杆菌表面展示系统因为芽孢的结构与特性，在作为口服递送载体时具有显著的优势：(1) 芽孢耐酸、耐高温等对于极端条件的抗性，使得芽孢作为口服递送载体具有很好的稳定性，能够在胃肠道环境中稳定存在而不会被破坏并将药物递送至指定部位[49]；(2) 现在广泛应用于益生菌制剂、膳食补充剂的几种芽孢杆菌具有极

表 2 芽孢杆菌表面展示系统的应用

Table 2 Applications of the surface display system of *Bacillus* spores

Type	Exogenous proteins/drugs	Anchoring proteins/attachment	Applications	References
Recombination	CsPmy	CotC	Oral vaccine for protection of grass carp against *Ascaris lumbricoides*	[42]
	Glucagon-like peptide-1 (GLP-1)	CotC	GLP-1 (28−36) oral delivery system for the treatment of type 2 diabetes mellitus	[35]
Non-recombinant	Grass carp reovirus (GCRV) Vp7 antigen	CotB & CotC	Enhancement of protective immunity to GCRV in grass carp	[43]
	OmpK or green fluorescence protein (GFP)	CotY	Enhancement of zebrafish immunogenicity to *Vibrio* pathogen infections	[44]
	Helicobacter pylori urease B	CotC	Oral administration of urease-carrying B spores prevents *Helicobacter pylori* infection	[45]
	Protective antigen (PA)	Adsorption	Development of an oral vaccine for the surface presentation of PA for the treatment of anthrax	[46]
	PfCSP	Coupling	Oral vaccines for malaria prevention	[47]
	TTFC	Adsorption	Effective in inducing an immune response and can be used as an adjuvant for vaccine immunization	[48]

高的安全性，这对口服递送载体是必需的[50]；(3) 芽孢的芽胞壳成分与融合的抗原不需要经过细胞壁的转位步骤就可以将重组细胞暴露在表面，克服了基于细胞的展示系统的尺寸限制问题[51]；(4) 基于芽孢外壳的紧密性和外壁的不必要性，一些大型蛋白可以展示在芽孢表面而不会影响芽孢的结构、抗性或萌发[36]；(5) 外源蛋白或多肽可以稳定地展示在芽孢表面，使酶能够更大程度地作用于底物，并且由于芽孢的抗性提高了融合蛋白在复杂环境中的活性与稳定性，增强免疫作用[52]。

重组和非重组芽孢杆菌表面展示系统也各自有其优缺点。重组型的一大明显优势是无需生产和纯化抗原，因为重组芽孢杆菌中包含与芽孢锚蛋白融合的抗原编码基因，所以芽孢杆菌细胞能够直接生产出需要展示的抗原，从而可以降低成本和简化生产过程[53]。

重组型的另一个优点是可以在同一芽孢表面上展示不同的抗原或使用表达单一抗原的芽孢混合物进行免疫。Dong 等[54]将幽门螺杆菌中性粒细胞激活蛋白(HP neutrophil-activiting protein, HP-NAP)和霍乱毒素 B (cholera toxin B, CTB)在枯草芽孢杆菌芽孢表面融合共表达，在研究中展现出了比单一蛋白表达载体更好的炎症抑制效果。非重组型的优点在于其较高的效率，Vetráková 等[55]使用枯草芽孢杆菌芽孢分别重组表面展示和吸附 SARS-CoV-2 穗状糖蛋白的受体结合域(receptor-binding domain, RBD)，而免疫荧光显微镜结果显示表面吸附RBD芽孢的荧光强度显著高于重组展示RBD芽孢，证实了非重组型的高效率。

此外，非重组型的另一个优势是其益于抗原以天然构象进行展示。大肠杆菌不耐热毒素(heat-labile enterotoxin B, LTB)是一种五聚体，其只有天然形式才具有活性功能，当 LTB 在芽孢表面以融合蛋白形式表达时只能以单体的形式展示，而通过吸附在芽孢表面表达时则可以以天然五聚体的形式展示[56]。重组型展示的成功取决于锚蛋白和目标蛋白的融合，所以重组型最大的局限性在于为特定的基因选择合适的锚蛋白。Hinc 等[57]报道了使用 CotB 和 CotC 作为锚蛋白展示金线莲 UreA 失败，而使用衣壳蛋白 CotG 为锚蛋白则展示成功。

非重组型的缺点在于芽孢展示的目标蛋白不在芽孢表面，只有芽孢在体内发芽或裂解后才能展示给免疫细胞；芽孢吸附的分子机制尚未完全明确，目标蛋白会无序地在芽孢表层积聚，从而影响载体精确结构的构建[53]。

2 酵母微胶囊系统

酵母是一类单细胞真菌。酵母及其生物制剂已经广泛应用在食品工业、生物医药等领域[58-59]。酵母微囊从酵母中提取，其成分主要是 β-葡聚糖，形状呈椭圆形，具有约 2−5 μm 的中空多孔结构[60] (图 2)。β-葡聚糖是酵母细胞壁的主要结构成分，在结构上主要由 β-1,3-葡聚糖的主链和 β-1,6-葡聚糖的侧链组成，其单糖组成为葡萄糖[61]。其中 β-1,3-葡聚糖既构成了葡萄糖刚性网络结构，也为酵母微囊提供了免疫活性[62]。β-1,3-葡聚糖是真菌的主要病原体相关分子模式，它能够被肠道免疫细胞通过多种模式识别受体(包括补体受体 3 和 Dectin-1 受体)识别出来，从而引起相关免疫反应[63]。

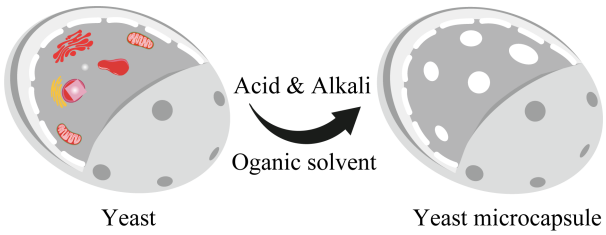

图 2 酵母微囊的制备与结构图

Figure 2 Preparation and structure of yeast microcapsules.

酵母微囊由β-葡聚糖构成了一个内部中空、表面多孔且带负电荷的结构，因此大多数的药物都可以在酵母微囊的内部被封装或者吸附在酵母微囊表面，有利于药物的递送[64]。随着研究的不断发展，酵母微囊的药物装载策略逐渐增多，根据刘瑛琪等[64]和 Wu 等[61]对酵母口服载体的综述，主要的药物装载策略有 5 种。

(1) 通过静电吸附作用将带正电的药物装载到酵母微囊内部。例如，Zhou 等[65]制备了带有正电荷的顺铂(cisplatin, CDDP)前体纳米粒(PreCDDP)，并将其封装到酵母微囊中，开发了一种安全有效的口服顺铂药物递送平台。Sabu 等[66]通过在酵母微囊表面负载带有相反电荷的胰岛素，并用海藻酸盐进行包被，成功实现了胰岛素的口服递送。

(2) 对于带负电荷的药物，可以通过非共价作用力修饰带正电荷的载体材料，再通过静电吸附作用装载到酵母微囊中。Feng 等[60]首先将小檗碱(berberine, BBR)、表没食子儿茶素没食子酸酯(epigallocatechin gallate, EGCG)和 Mn^{2+}通过非共价分子间作用力自组装成纳米颗粒(BBR/MPN NPs)，而后进一步装载到酵母微囊中。王大壮[67]将 DOX 装载在 3-巯基丙酸(3-mercaptopropionic acid, MPA)修饰的纳米氧化锌(ZnO NPs)上，构建了带负电荷的载药纳米粒(MPA-ZnO-DOX)，再用聚乙烯亚胺(polyethyleneimine, PEI)将酵母微囊内部修饰成正电荷，使 MPA-ZnO-DOX 能够装载在酵母微囊中。

(3) 可以利用被动扩散或疏水作用将不带电的药物修饰进酵母微囊中。Shi 等[68]通过被动扩散将牛血清蛋白(bovine serum albumin, BSA)包封在被壳聚糖包被的酵母微囊里。除此之外，咖啡因、维生素 B_{12} 等都可以通过相互孵育利用被动扩散装载到酵母微囊中[69]。

(4) 部分药物还可以通过逐层合成(LBL)进行装载。Tan 等[70]通过静电相互作用将带有相反电荷的多糖依次沉积在预先装载花青素的酵母微囊上，制备出用于花青素包封和稳定的口服递送载体。He 等[71]将替莫唑胺(temozolomide, TMZ)装载在聚乳酸-羟基乙酸共聚物(poly(lactic-co-glycolic acid), PLGA)中，并通过逐层合成依次包裹 O^6-苄基鸟嘌呤(O^6-benzylguanine, O^6-BG)接枝的壳聚糖(BG-chitosan, BG-CS)层和酵母微囊，最终获得了一个稳定的 TMZ 口服递送平台。

(5) 某些药物还可以将其与衍生化的酵母微囊进行结合。Soto 等[72]使用原位合成酵母微囊包覆镍纳米颗粒，将带有 His-tag 的 Cda2 蛋白通过非共价作用力装载进酵母微囊中。Pan 等[73]使用 $NaIO_4$ 对酵母微囊进行氧化，使其具有醛基，进而与卵清蛋白(ovalbumin, OVA)的伯胺基反应，将 OVA 通过化学连接到酵母微囊表面，从而在体内产生有效的特异性免疫反应。

酵母微囊作为口服递送载体具有诸多优势。首先，因为人体胃肠道中缺乏可以降解 β-葡聚糖的酶，而且 β-葡聚糖具有耐酸的特性，所以以β-葡聚糖为主要成分的酵母微囊可以避免在恶劣的肠道环境中被消化降解，保护药物可以顺利通过复杂的肠道环境而不被破坏[60-61,74]。其次，β-葡聚糖是一种来源于酵母细胞的载体，因此可以模仿酵母的行为，被 M 细胞上的 Dectin-1 识别，从而通过复杂的巨噬细胞介导运动，将各种药物准确、安全地靶向地送到病变部位[75-76]。

酵母微囊对极性和非极性的小分子具有良好的渗透性，而且可以通过调节表面紧密连接程度促进对生物大分子的吸收，所以可以有效地装载各类大分子和小分子的药物，例如蛋白质、DNA、抗原、姜黄素等[63,68,77]。最后，酵母微囊具有无细胞毒性以及安全的特点，这是作为口服递送载体的一大根本要求[78-79]。

3 重组益生菌表达系统

益生菌具有安全、可以进行基因工程改造的特点，伴随着分子生物学和基因工程技术的不断发展，现在工程化益生菌已经广泛应用于各类工业生产中[80-81]。在此前的研究中，重组益生菌是通过基因编辑等技术将特定的基因重组进益生菌中，使得益生菌可以表达所需要的特定产物[82]。随着对重组益生菌研究的不断深入，许多种属的益生菌已经被研究改造成各类外源基因的重组表达载体，除了前文提到的芽孢杆菌、酵母等，岳梦云[83]将乳酸乳球菌改造成能够表达胰高糖素样肽-1 (glucagon-like peptide-1, GLP-1)蛋白的载体，用以治疗帕金森病；赵瑞[84]工程化大肠杆菌 Nissle1917 菌株，使其能够异源表达尿酸代谢基因和提高氧气利用率基因，以实现高效地降解尿酸；Mathipa-Mdakane 等[85]还综述了鼠李糖乳杆菌作为工程益生菌的应用。

用作重组载体的益生菌的种类繁多，不同种类的重组益生菌表达外源蛋白的方式也不尽相同。乳酸菌是一种具有非致病性和非定殖性的益生菌，已被详细研究且越来越多地被用作外源蛋白的生产宿主[86]。重组乳酸菌所携带的外源蛋白可以以 3 种形式表达，包括在细胞质中表达、锚定在细胞壁上表面展示和分泌到细胞外部[87] (图 3)。Muñoz 等[88]以乳酸乳球菌为载体，构建了能在大西洋鲑体内生产和释放I型干扰素(interferon-la, IFN-la)的工程益生菌，并测定发现 IFN-la 在细胞质中表达。Huang 等[89]通过将非洲猪瘟病毒(African swine fever virus, ASFV)编码的 p14.5 基因插入到载体质粒 pLP-S 中，使 p14.5 蛋白成功锚定在植物乳杆菌的 S 层蛋白中，从而将 p14.5 蛋白表面展示在植物乳杆菌上。Zhao 等[90]克隆了来自嗜水气单胞菌的 Aha1 基因，并使其在干酪乳杆菌中分泌表达，而口服重组干酪乳杆菌的鲤鱼体内抗体水平显著提高。更多关于不同种益生菌表达外源蛋白的附加信息总结在表 3 中。

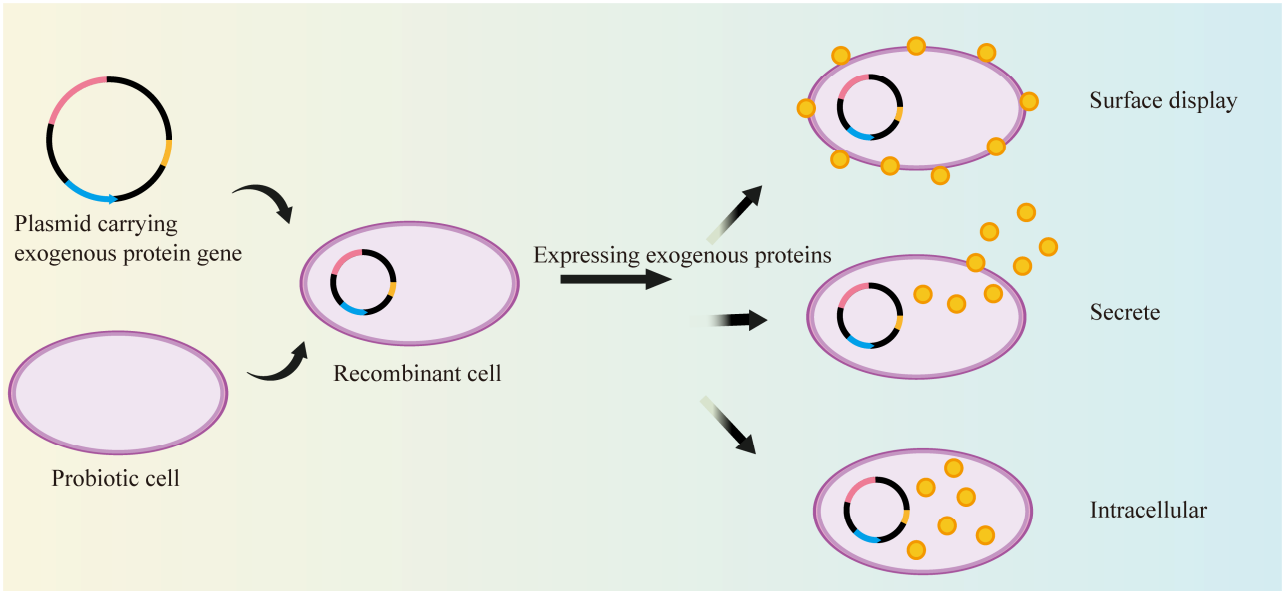

图 3　重组益生菌表达外源蛋白的不同方式

Figure 3　Different ways of expressing exogenous proteins by recombinant probiotics.

表 3　不同种益生菌表达外源蛋白

Table 3　Expression of exogenous proteins by different species of probiotics

Protein expression mode	Bacterial strain type	Applications	References
Surface display	*S. cerevisiae*	Expression of avian influenza HA protein for use as an oral vaccine against avian influenza	[91]
	L. plantarum	Expression of *Clostridium nucleatum* outer membrane FomA protein inhibits IBD	[92]
	L. plantarum	Expression of *Staphylococcus aureus* non-toxic mutant alpha hemolysin, protective against *Staphylococcus aureus* lung and skin infections	[93]
	E. faecalis	Separate expression of four *Eimeria aphidis* proteins of Tennessee and protection against homologous infection in chickens by combined immunization	[94]
	Bifidobacterium	Expression of WT1 protein for use as an oral vaccine against oral cancer	[95]
	L. plantarum	Expression of the influenza virus antigen HA1-DCpep, which can play an immunizing role against influenza virus	[96]
	L. casei	Expression of AHA1-CK6 and VP2 proteins and oral immunization induces prevention of infectious pancreatic necrosis in rainbow trout	[97]
	L. casei	Expression of DCpep, Col, and CoE antigenic fusion proteins effectively induces an immune response against porcine epidemic diarrhea virus	[98]
	L. casei	Expression of an OmpK fusion CTB protein for use as an adjuvant against *Vibrio* anisopliae infection in crucian carp	[99]
Secrete	*P. pentosaceus*	Secretion of therapeutic protein P8 for tumor suppression and regulation of intestinal microflora	[100]
	L. paragasseri	Express and efficiently secrete epitope-4	[101]
	B. subtilis	Expression of the multi-epitope protein OmpC-FliC-SopF-SseB-IL-18 induces a strong immune response against *Salmonella enteritidis*	[102]
	L. lactis	Expression of extracellular TGFβR2 with significant anti-hepatic fibrosis effects	[103]
	L. lactis	Expression of soluble CD80 enhances anti-tumor immunity to inhibit tumor growth	[104]
Intracellular	*L. casei*	Expression of an antigen directed against SARS-CoV-2 Omicron variant B.1.1.529	[105]
	L. lactis	Expression of heat shock protein 65 and inhibition of atherosclerosis	[106]
	L. lactis	Expression of *Helicobacter* lipoprotein Lpp20 produces significant immune effects	[107]

不同的外源蛋白表达方式也各有其优势。在细胞质中表达的优点在于可以避免外源蛋白在复杂的胃肠道环境中被水解破坏,因为只有当宿主菌株裂解之后外源蛋白才会被释放出来[108]。Yang 等[109]构建了能够在细胞质内共表达 SO7 和 DCpep 基因的植物乳杆菌重组载体，与对照组相比展现出显著的抗柔嫩艾梅尔球虫感染的效应。锚定在细胞壁上表面展示的优点是使得外源蛋白与细胞紧密地结合，充分地与黏膜接触，显著增强机体免疫反应,研究表明锚定在细胞壁上表面展示的外源蛋白通常可以激发出更强的宿主免疫反应[91]。Ai 等[110]构建出在细胞不同位

置表达 Der p2 的重组乳酸乳球菌表达载体，其中在细胞壁锚定的 E7 抗原免疫效果优于表达在细胞质内 E7 抗原的免疫效果。

如果将合成的外源蛋白分泌到细胞外部，则可以避免外源蛋白在细胞质内水解和外源蛋白积累对宿主细胞的毒性[111]。Ren 等[112]通过在乳酸乳球菌的不同位置表达出花生过敏原 Ara h2，发现将抗原分泌到细胞外部的疫苗的免疫调节能力显著高于其他两种方式。

重组益生菌表达外源蛋白的方式对外源蛋白的活性及其数量有明显的影响，但是不同方式各有其优劣势。在实际生产应用中应结合具体条件，应综合考虑外源蛋白类型与结构、菌株种类等因素，从而选择合适的益生菌。

4 新兴的口服递送载体：细菌样颗粒和细菌影

除了上述提到了几种常见的口服递送载体之外，随着研究的不断发展，现在也出现了一些新兴的口服递送载体，下面简述两种新兴的口服递送载体：细菌样颗粒(bacterium-like particles, BLPs)表面展示系统和细菌影(bacterial ghosts, BGs)。

4.1 细菌样颗粒(BLPs)

BLPs 是一种将乳酸菌经过热酸处理，使得除乳酸菌的刚性细胞壁肽聚糖(peptidoglycan，PGN)之外的成分，包括 DNA、蛋白质、细胞壁等全部破坏，从而产生的无活性、与活菌形状相似的空心颗粒，也被称为革兰氏阳性菌增强基质(Gram-positive enhancer matrix, GEM)[113]。BLPs 表面展示系统包括 3 个部分：BLPs 作为支架单元、锚定蛋白(protein anchor, PA)作为锚定单元和目标蛋白作为活性单元。目标蛋白和锚定蛋白 PA 在体外融合表达后，通过锚定蛋白 PA 紧密结合在热酸处理后的乳酸菌肽聚糖表面，实现目标蛋白的表面展示[114] (图 4)。

BLPs 表面展示系统具有许多的优势：(1) 无安全顾虑且性质稳定，可在室温下贮存；(2) 目标蛋白与 BLPs 的结合紧密，稳定性高；(3) 较乳酸菌活菌可以更高密度地装载目标蛋白；(4) 可以诱导全身和黏膜免疫，用作免疫佐剂；(5) 可以高效地同时展示多种抗原；(6) 展示和传递目标蛋白的数量已知且恒定[115-117]。Liu 等[118]使用 GEM 颗粒和重组抗原 CTB-UE 构成了口服疫苗 CUE-GEM，并证实其可以增强抗幽门螺旋杆菌感染的天然免疫反应。Mao 等[119]将单链胰岛素 SCI-59 与乳酸菌 BLPs 颗粒结合，口服后可诱导非肥胖糖尿病(non obese diabetes, NOD)小鼠的口服耐受性并预防自身免疫性糖尿病，其效果与稳定性还优于直接使用 SCI-59。

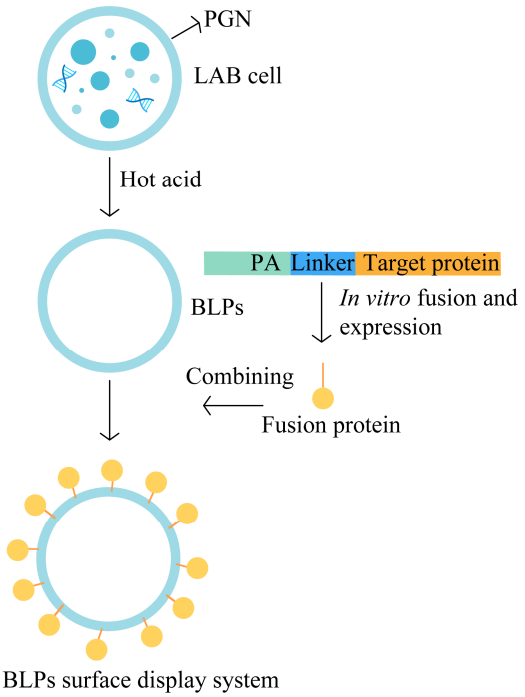

图 4 BLPs 表面展示系统的制备过程

Figure 4 Preparation process of BLPs surface display system.

然而 BLPs 表面展示系统也存在着一些问题：有报道发现以 BLPs 作为载体的疫苗一次免疫的效果并不明显，通常需要 2 到 3 次免疫才能有一定的效果[120]。Sudo 等研究发现 BLPs 递送的效果与递送途径有关，而滴鼻途径的效果高于口服途径[121]。目前，关于 BLPs 表面递送的研究主要集中在滴鼻递送方面，而关于口服递送方面的研究还不充足。

4.2 细菌影(BGs)

BGs 是通过基因或化学方法产生的非生物空包膜，常见的是革兰氏阴性细菌，如大肠杆菌 Nissle1917 受到裂解基因 E 控制表达，在活菌包膜内形成裂解隧道结构[122-123] (图 5)。BGs 不含有任何细胞质内容物，包括染色体和质粒 DNA，但保留了其细胞表面形态结构：外膜、内膜和肽聚糖。因为 BGs 保留了完整的细胞形态和天然表面抗原结构，如病原体相关分子模式，其中包括天然细菌的脂多糖、鞭毛、黏附素等与免疫相关的刺激因子，所以 BGs 易于被免疫细胞，如巨噬细胞、B 和 T 细胞等识别和捕获，能够有效地激发强大的免疫反应，无需添加额外的佐剂，表明 BGs 具有良好的作为疫苗的潜力[124-125]。

BGs 的空洞包膜的结构，使其非常适合用作各种药物的载体。药物可以锚定在 BGs 的细胞内膜和外膜中，也可以锚定和填充在 BGs 的周质空间和内腔中，其载药机理是药物与内部空间之间的简单静电相互作用[126-127]。Zhu 等[128]成功将埃博毒素 B 通过非共价作用力装载到由 *EcN* 制备的 BGs 的内部空间中，并可以显著提高埃博毒素 B 在 HeLa 细胞中的抗癌效果，并减少所需的剂量和相关的副作用。因为 BGs 是一种非活体细菌，内部不含任何有害化学物质，也不会产

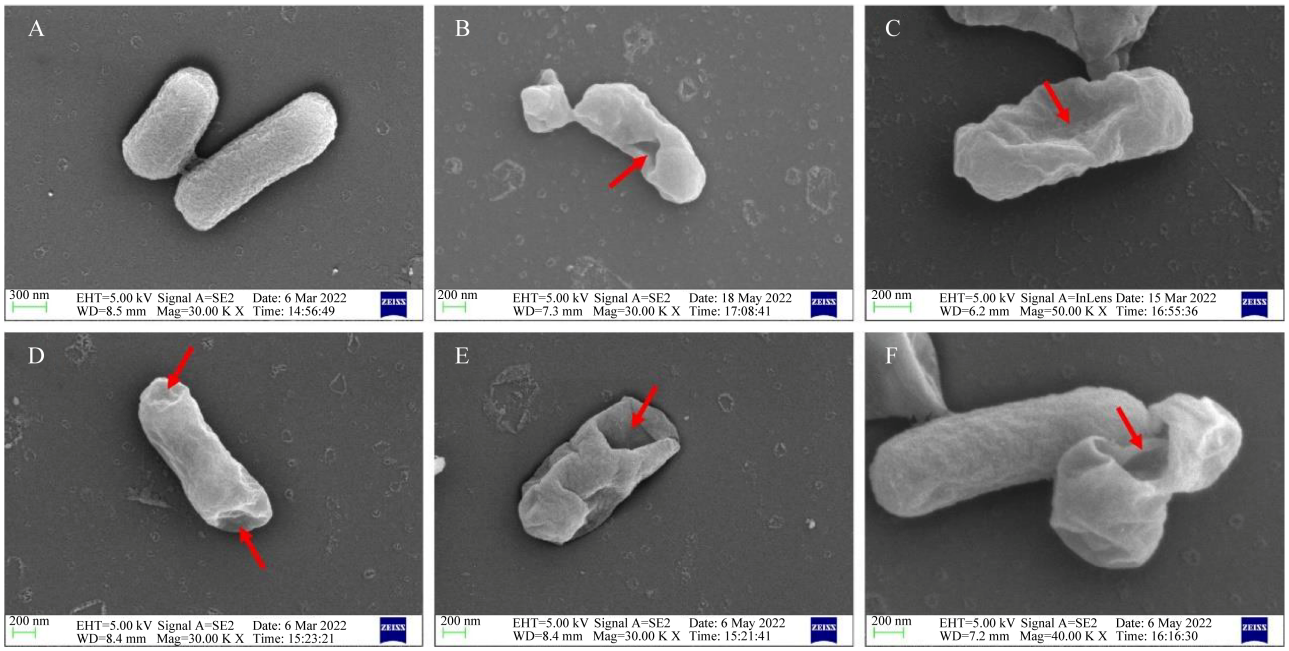

图 5 扫描电镜观察大肠杆菌 BGs 的形态 A：野生型大肠杆菌的完整形态. B−F：诱导裂解形成的大肠杆菌 BGs. 红色箭头表示跨膜隧道[124]

Figure 5 Morphology of *Escherichia coli* BGs observed by scanning electron microscopy. A: Intact morphology of wild type *E. coli*. B−F: *E. coli* BGs formed by induced lysis. Red arrows indicate transmembrane tunnels[124].

生细胞毒性和恢复到致病形式以造成危害，而且 BGs 是无 DNA 的，也不存在基因水平传播的风险，所以 BGs 具有很高的安全性[129]。

此外，BGs 在室温下具有优异的稳定性，也表明其是一种很好的候选口服递送载体[130]。Eslaminejad 等[131]用干酪乳杆菌制备了一种干酪乳杆菌 BGs 用以装载克霉唑，并展现出优异的载药能力和显著的抗念珠菌作用。谢松志[132]将抗菌药物 CIP 载入到 *EcN* 制备的 BGs 内腔中，具有靶向和刺激响应性释放药物的能力，成功构建出一种新型口服载药平台。然而，有些报道也指出了 BGs 存在的问题：BGs 的空洞结构会使得靶点药物浓度和免疫原性下降；BGs 似乎不适合免疫力低下的病人[133]。尽管如此，BGs 是一种很有前景的口服递送载体，但是对于其的研究还有很多工作要做。

5 总结与展望

益生菌是理想的口服递送载体，可以保护药物顺利通过复杂胃肠道环境而不被破坏，能够将药物靶向递送到特定病变部位，并且具有很好的安全性和与药物结合的稳定性。因此许多益生菌已经被开发为递送各种药物的疫苗，如 DNA 疫苗、蛋白质疫苗、化学药物疫苗和多肽疫苗等[134-137]，并且应用在治疗过敏、炎症、肿瘤等疾病中[138-140]。

本文综述了 3 种常见的益生菌口服递送载体和 2 种新兴的口服递送载体，并介绍了它们的结构与特点。益生菌的特殊结构，如芽孢杆菌的芽孢、酵母的 β-葡聚糖等可以很好地装载药物并保护它们通过复杂的肠道环境，以实现药物的高效递送，并且益生菌口服递送载体具有表面展示、分泌、在细胞质内等多种药物表达模式，可以根据药物的种类和特点进行匹配。此外，可以用作口服递送载体的益生菌种类繁多，包括乳酸菌、酵母、益生芽孢杆菌和大肠杆菌 Nissle1917 等，具有广泛的开发潜力。益生菌口服递送载体以其特殊的结构提高药物递送的稳定性与靶向性，多种药物表达模式为药物的选择使用提供普遍性，再佐以益生菌安全无害的特性，是一种非常有应用潜力的药物递送系统。

然而，益生菌口服递送载体目前仍然存在以下问题。许多益生菌口服递送载体是使用非活性细菌制备的，而制备过程的研究与安全控制方面的研究还不充分，使得益生菌口服疫苗的工业化生产存在阻碍。此外，使用益生菌作为递送载体的最大问题就是其在胃肠道中的稳定性，口服益生菌普遍存在生物利用度低和治疗效果不稳定的问题[141]。针对这些问题，目前也有很多研究聚焦于在益生菌表面修饰各种涂层以达到保护益生菌的目的，包括物理修饰和化学修饰等[142-144]。

最后，现在对于益生菌口服递送载体的研究更多是在动物模型上实现的，在临床上的报道较少。益生菌口服递送载体是一种很有应用前景的药物递送平台，相信随着研究的不断深入与丰富，其可以在药物递送领域提供一条可靠的选择。

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